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Detection of Antibodies to E2-Protein of GB Virus-C/Hepatitis G Virus in Patients With Acute Posttransfusion Hepatitis

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GB virus-C/hepatitis G virus (GBV-C/HGV) is an RNA virus that can be transmitted by transfusion with the diagnosis based on the detection of serum GBV-C/HGV RNA by reverse transcription–polymerase chain reaction. In order to evaluate the role of antibodies to the E2 protein (anti-E2) of GBV-C/HGV in patients with acute posttransfusion hepatitis, anti-E2 was measured in one patient with acute GBV-C/HGV infection, five patients with acute GBV-C/HGV and hepatitis C virus (HCV) coinfection, and four patients with positive pretransfusion GBV-C/HGV RNA who were superinfected with HCV after transfusion. One patient with acute GBV-C/HGV infection remained GBV-C/HGV RNA-positive 18 months after transfusion and did not develop anti-E2. Among five patients with acute GBV-C/HGV and HCV coinfection, one lost GBV-C/HGV RNA 28 months after transfusion and developed anti-E2 independent of serum alanine aminotranferase levels. The other four patients remained GBV-C/HGV RNA-positive and anti-E2–negative at the end of follow-up. Among four patients with positive pretransfusion GBV-C/HGV RNA and superinfected with HCV, one lost GBV-C/HGV RNA 2 months and one 10 months after superinfection and subsequently developed anti-E2. The other two patients remained GBV-C/HGV RNA-positive without anti-E2 at the end of follow-up. Sixty-five samples tested were mutually and exclusively positive for either GBV-C/HGV RNA or anti-E2; only one sample was positive for both GBV-C/HGV RNA and anti-E2. In conclusion, the development of anti-E2 of GBV-C/HGV usually indicates the clearance of serum GBV-C/HGV RNA in patients with acute posttransfusion hepatitis. ***J. Med. Virol. 57:85–89, 1999.***

KEY WORDS: GBV-C/HGV; GBV-C/HGV RNA; anti-E2 of GBV-C/HGV; acute posttransfusion hepatitis

INTRODUCTION

After the cloning of hepatitis C virus (HCV) in 1989, hepatitis A to E viruses accounted for more than 90% of patients with posttransfusion hepatitis. In 1995, a new transfusion-transmissible agent, GB virus-C/hepatitis G virus (GBV-C/HGV), was cloned by two independent laboratories in the United States [Simons et al., 1995a, 1995b; Leary et al., 1996; Linnen et al., 1996]. The infection is currently diagnosed by the presence of serum GBV-C/HGV RNA measured by reverse transcription–polymerase chain reaction (RT-PCR) [Schlueter et al., 1996; Bhardwaj et al., 1997; Cantaloube et al., 1997]. In prospective posttransfusion studies in the United States and Taiwan before the screening of blood donors with antibody to HCV (anti-HCV), 70–80% of patients with posttransfusion hepatitis were due to HCV infection, 10–20% were due to coinfection of HCV and GBV-C/HGV, and 23–30% of posttransfusion non-A–E hepatitis were caused by GBV-C/HGV infection [Lee et al., 1991a; Linnen et al., 1996; Alter HJ et al., 1997; Hwang et al., 1997].

Since only a few cases of acute posttransfusion GBV-C/HGV infection have been identified by prospective follow-up studies, the natural course of infection remains to be clarified. However, most reported cases showed spontaneous resolution of hepatitis [Alter HJ et al., 1997; Hwang et al., 1997]. Whether these cases developed antibody to GBV-C/HGV is of clinical interest. Specific immunoassays are required to follow the acute course of GBV-C/HGV infection and to indicate whether antibody to GBV-C/HGV develops or viremia persists. Recently, an ELISA that detects the antibodies to the E2 protein (anti-E2) of GBV-C/HGV was developed by Tacke et al. [1997]. The purpose of this

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study was to test the presence of anti-E2 in patients with acute posttranfusion hepatitis and to evaluate the relationship between the presence of serum GBV-C/HGV RNA and the development of anti-E2.

MATERIALS AND METHODS

Patients With Posttransfusion Hepatitis

One patient with acute GBV-C/HGV infection only, five patients with acute GBV-C/HGV and HCV coinfection, and four patients with positive pretransfusion GBV-C/HGV RNA, who were superinfected with HCV after transfusion, were recruited from a prospective posttransfusion study in Taiwan [Lee et al., 1991a, 1991b; Hwang et al., 1997]. Among these patients, pretransfusion and serial posttransfusion blood samples were available for both GBV-C/HGV RNA and anti-E2 measurements. Serum samples were stored in aliquots at -70°C until analyzed.

Laboratory Tests

For GBV-C/HGV RNA determination, nucleic acid was first extracted from 125 μL of serum using PureScript (Gentra Systems, Minneapolis, MN). Reverse transcription was then carried out with random hexamer and Superscript (Life Technologies, Grand Island, NY). Nested-PCR with 30 cycles and 35 cycles of amplification was followed in a PCR machine (GeneAmp PCR System 2400, Roche Diagnosis Systems, Basal, Switzerland). The primers used were from the NS-5 region of the HGV genome: outer primers (sense, 5′-CTGATGTTGCTAGCCTGTGTGAGA-3′, and antisense, 5′-ACCGACACCTTAGATCCCCAGCCC-3′) and inner primers (sense, 5′-AGAACCATACAGCCTATTGTGACC-3′, and antisense, 5′-CCTTACAGTCCTTATTGCTTCCTC-3′). The amplified product (460 base pairs) was visualized under ultraviolet light after gel electrophoresis and stained with ethidium bromide. Positive RNA control from Genelabs Technologies (Redwood City, CA) was used in each assay. GBV-C/HGV RNA was assessed in duplicate sera and reported as positive only when consistent results were obtained.

Anti-E2 of GBV-C/HGV was measured using "μ Plate Anti-HGenv" (Boehringer Mannheim, Germany) according to the protocol provided by the manufacturer [Tacke et al., 1997]. Briefly, a DNA fragment encoding the putative E2 transmembrane protein of GBV-C/HGV was amplified and verified by DNA sequencing. The antigenic fusion protein was stably expressed under a cytomegalovirus promoter within Chinese hamster ovary (CHO) cells. The recombinant E2-antigen was bound via the biotin-conjugated anti-E2 monoclonal antibody (mouse) onto a streptavidin-coated microtiter plate. Plates were incubated with the diluted human specimen, specifically, bound antibodies from the human IgG-peroxidase conjugate, and ABTS as peroxidase subtract. Results were analyzed according to optical density (OD) values measured at 405 nm compared to the cutoff value with the help of kit-specific positive and negative controls. To ensure specificity, serum samples with borderline OD values near the cutoff value were retested in parallel on negative control plates, which had been incubated without recombinant E2-antigen (confirmation test). Sera were considered positive for anti-E2 by a maximal reduction factor of 0.66 of the OD values in the assay without E2-protein compared to the assay with E2-antigen.

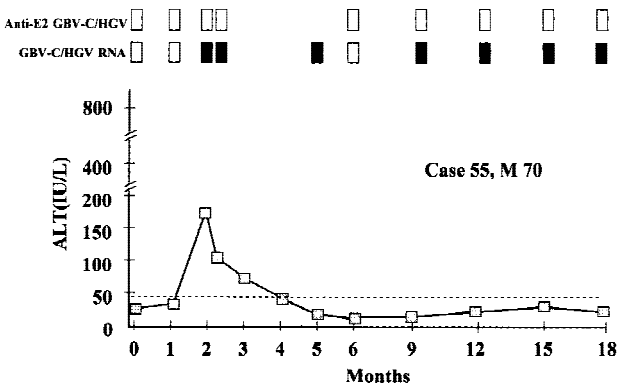

Fig. 1. Clinical pictures of a patient with acute posttransfusion GBV-C/HGV infection. GBV-C/HGV RNA was negative in pretransfusion sera, converted to positive 2 months after transfusion and remained positive up to the end of follow-up. Anti-E2 of GBV-C/HGV remained negative after 18 months follow-up. ■: positive, □: negative.

Anti-HCV was measured by a second-generation enzyme immunoassay (Abbott Laboratories, Chicago, IL). HCV RNA was first measured using branched-chain DNA signal amplification (bDNA) assay (Quantiplex 2.0, Chiron, Emeryville, CA) and was further analyzed by RT-nested PCR if the bDNA assay showed a negative detection as in previous reports [Hwang et al., 1993; Chan et al., 1995].

RESULTS

One patient with acute GBV-C/HGV infection remained serum GBV-C/HGV RNA-positive 18 months after transfusion without anti-E2 (Fig. 1). Further serum samples were not available due to lack of follow-up. This patient resolved hepatitis spontaneously and serum alanine aminotransferase (ALT) returned to normal 4 months after transfusion. Among five patients with acute GBV-C/HGV and HCV coinfection, four developed chronic hepatitis. One patient was negative for GBV-C/HGV RNA 28 months after transfusion, and anti-E2 was developed 24 months after transfusion and persisted after disappearance of GBV-C/HGV RNA (Fig. 2). However, the development of anti-E2 was unrelated to serum levels of ALT. The patient progressed to chronic hepatitis, and serum HCV RNA and anti-HCV were positive at the end of follow-up. The other four patients remained GBV-C/HGV RNA-positive and anti-E2–negative at the end of follow-up (median: 15 months after transfusion). Serum HCV RNA and anti-HCV remained positive at the end of follow-up in three patients who developed chronic hepatitis. In the patient with resolved hepatitis, serum

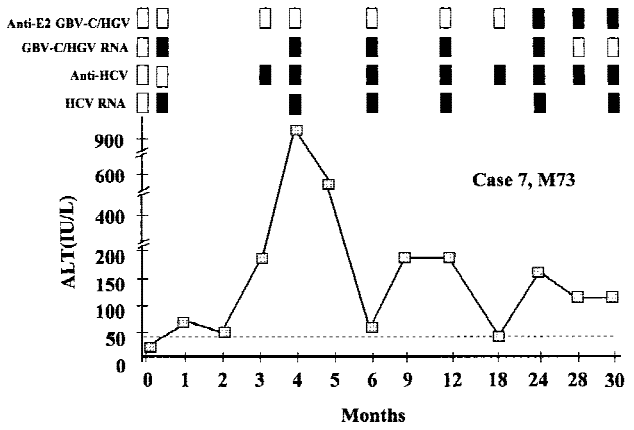

Fig. 2 Clinical pictures of a patient with acute posttransfusion GBV-C/HGV and HCV coinfection. HCV RNA and GBV-C/HGV RNA were negative in pretransfusion sera and both converted positive 2 weeks after transfusion. GBV-C/HGV RNA was negative after 28 month follow-up, and anti-E2 of GBV-C/HGV developed after 24 months follow-up. Serum anti-HCV and HCV RNA remained positive up to the end of follow-up. ■: positive, □: negative.

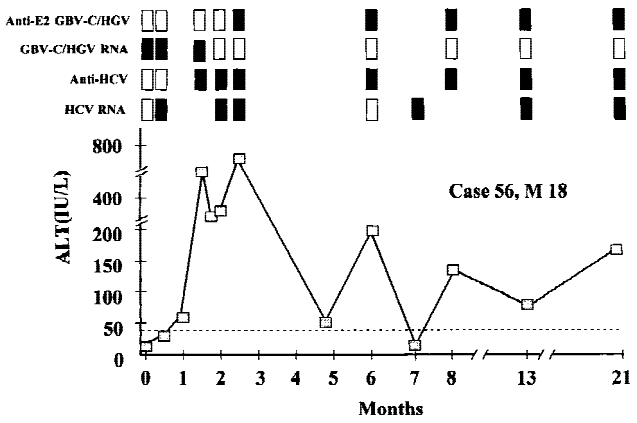

Fig. 3. Clinical pictures of a patient who had pretransfusion sera positive for GBV-C/HGV RNA and developed chronic hepatitis after superinfection with HCV. Serum HCV RNA and anti-HCV remained positive up to the end of follow-up. Serum GBV-C/HGV RNA was positive in pretransfusion sera and became negative 2 months after HCV superinfection. Anti-E2 of GBV-C/HGV developed 10 weeks after transfusion. ■: positive, □: negative.

HCV RNA disappeared 6 months after transfusion but anti-HCV disappeared 21 months after transfusion.

Among four patients with positive pretransfusion GBV-C/HGV RNA and superinfected with HCV, one patient was negative for GBV-C/HGV RNA 2 months and one 10 months after transfusion. Both subsequently developed anti-E2 that was unrelated to serum levels of ALT (Fig. 3). The first patient described progressed to chronic hepatitis, and serum HCV RNA and anti-HCV were positive at the end of follow-up. The other patient resolved hepatitis. Serum HCV RNA was negative 4 months after transfusion but anti-HCV was still positive at the end of follow-up (66 months after transfusion). The other two patients remained GBV-C/HGV RNA-positive and anti-E2–negative at the end of follow-up (18 months and 23 months after transfusion, respectively). These two patients progressed to chronic hepatitis with positive serum HCV RNA and anti-HCV at the end of follow-up.

Among 70 samples tested for both GBV-C/HGV RNA and anti-E2, 65 samples were mutually exclusive positive for either GBV-C/HGV RNA or anti-E2. Only one sample was positive for both GBV-C/HGV RNA and anti-E2 and four samples were negative for both GBV-C/HGV RNA and anti-E2 simultaneously.

DISCUSSION

GBV-C/HGV was cloned in 1995 and was identified as a transfusion-transmissible agent [Simons et al., 1995a, 1995b; Leary et al., 1996; Linnen et al., 1996]. However, the clinical course of acute posttransfusion GBV-C/HGV infection is not yet clear due to the limited number of cases reported. Also, the role of GBV-C/HGV infection in chronic hepatitis is questionable due to a similar prevalence of GBV-C/HGV in patients with non-A–E chronic hepatitis and in those coinfected with hepatitis B or C. There is also no effect of GBV-C/HGV on the clinical course of chronic hepatitis C in patients coinfected with GBV-C/HGV and HCV [Tanaka et al., 1996; Alter MJ et al., 1997; Bralet et al., 1997; Francesconi et al., 1997; Martinot et al., 1997; Saiz et al., 1997; Pawlotsky et al., 1998]. In addition, the role of GBV-C/HGV in fulminant hepatitis is also questionable due to discordant results from different countries [Yoshiba et al., 1995; Sallie et al., 1996]. An understanding of the clinical course of acute GBV-C/HGV infection and the relationship between serum GBV-C/HGV RNA and antibody to GBV-C/HGV antigens may help answer some of these questions.

A viral protein, E2, is located on the surface of the virus and is presumed to be the target for humoral immune response. Previous studies showed that antibodies to prokaryotically expressed GBV-C recombinant proteins showed no consistent results [Dawson et al., 1996]. However, recent development of a putative E2 envelope protein of GBV-C/HGV was cloned and expressed in a eukaryotic cell system [Pilot-Matias et al., 1996; Dille et al., 1997; Tacke et al., 1997]. The secreted E2 protein was purified for a solid-phase ELISA. According to recent epidemiological studies in the Western countries, anti-E2 was present in 3–9% of blood donors, 13–21% of patients on hemodialysis, and 41–85% of intravenous drug users, while the GBV-C/HGV RNA prevalence rate was 1.0–2.5%, 9–10%, and 3.7–38%, respectively [Dille et al., 1997; Gutierrez et al., 1997; Hassoba et al., 1997; Nubling et al., 1997; Tacke et al., 1997; Ross et al., 1998]. The presence of anti-E2 and GBV-C/HGV RNA was mutually exclusive in most cases: none of the blood donors and patients on hemodialysis, and 0–12% of the drug users, were positive for both markers at the same time.

In our study, we evaluated the relationships of serum GBV-C/HGV RNA and anti-E2 in patients with acute posttransfusion hepatitis. Our results showed that anti-E2 developed after the disappearance of se-

rum GBV-C/HGV RNA in one patient with acute GBV-C/HGV and HCV coinfection and in two patients who had pretransfusion GBV-C/HGV infection and who were superinfected with HCV after transfusion. In those patients who remained serum GBV-C/HGV RNA-positive, none developed anti-E2. Among 70 samples tested for both GBV-C/HGV RNA and anti-E2, 65 samples were mutually exclusive positive for either GBV-C/HGV RNA or anti-E2. Only one sample was positive for serum GBV-C/HGV RNA and anti-E2 simultaneously. These findings confirm the link between development of anti-E2 and resolution of GBV-C/HGV infection. In addition, the development of anti-E2 in patients with acute GBV-C/HGV and HCV coinfection and GBV-C/HGV with HCV superinfection showed no relationship to serum levels of ALT or the clinical course of patients.

In conclusion, the results confirm and extend previous findings [Pilot-Matias et al., 1996; Dille et al., 1997; Gutierrez et al., 1997; Tacke et al., 1997] that GBV-C/HGV anti-E2 is associated with loss of GBV-C/HGV RNA and also is a marker for recovery from GBV-C/HGV infection.

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